Recovery from stress is a function of age and telomere length

Graham M. Strub · Amy Depcrynski ·
Lynne W. Elmore · Shawn E. Holt

Received: 30 January 2008 / Revised: 7 April 2008 / Accepted: 14 April 2008 / Published online: 20 May 2008
© Cell Stress Society International 2008

Abstract Cells are constantly exposed to a wide variety of stimuli and must be able to mount appropriate physiological responses in order to maintain proper form and function. Cells from every organism have evolved highly conserved mechanisms to cope with environmental changes, including the widely studied heat shock response (HSR), which is induced by a variety of cellular stresses such as heavy metal ion exposure. It has long been known that as organisms and individual cells age, their ability to appropriately cope with

environmental stress is attenuated. Here, we examine the ability of two heavy metal ions (ZnCl_2 , SnCl_2) to induce the HSR in human fibroblasts by assessing the expression of heat shock proteins (Hsp90, Hsp70, and p23) and the ability of the cells to recover over time. We demonstrate that the induction and recovery of chaperone levels is attenuated with age and that cells immortalized with the human telomerase reverse transcriptase component of the telomerase enzyme do not attenuate their HSR as their replicative age increases. Our data suggest that the recovery of normal human cells from an HSR is related in part to age and the cell's overall telomere length.

G. M. Strub
Department of Biochemistry, Medical College of Virginia Campus
at Virginia Commonwealth University,
1101 E. Marshall St.,
Richmond, VA 23298-0662, USA

A. Depcrynski · S. E. Holt
Department of Human and Molecular Genetics, Medical College
of Virginia Campus at Virginia Commonwealth University,
1101 E. Marshall St.,
Richmond, VA 23298-0662, USA

L. W. Elmore · S. E. Holt (✉)
Department of Pathology, Medical College of Virginia Campus
at Virginia Commonwealth University,
1101 E. Marshall St.,
Richmond, VA 23298-0662, USA
e-mail: sholt3@mcvh-vcu.edu

S. E. Holt
Department of Pharmacology and Toxicology, Medical College
of Virginia Campus at Virginia Commonwealth University,
1101 E. Marshall St.,
Richmond, VA 23298-0662, USA

L. W. Elmore · S. E. Holt
Massey Cancer Center, Medical College of Virginia Campus
at Virginia Commonwealth University,
1101 E. Marshall St.,
Richmond, VA 23298-0662, USA

Keywords Aging · Stress · Hsp90 · Chaperones · Hsp70 · p23 · Telomere

Introduction

Aging is a multicausal process leading to the gradual decay of self-defense mechanisms and an accumulation of damage, resulting in a diminished capability to maintain homeostasis and an increased vulnerability to stresses. Many theories of aging are stochastic, such as the accumulation of DNA damage (Failla 1958), error catastrophe and accumulation of misfolded proteins (Levine and Stadtman 1996), and exposure to oxidative stress (Sohal and Weindrich 1996). These theories suggest that the accumulation of damage over time leads to a decreased ability for organisms and cells to cope with environmental changes. Other theories are “preprogrammed” based on the fact that there is clearly a genetic component to aging with many genes having been identified that contribute to the aging process (Finch and Tanzi 1997; Jazwinski 1996; Murakami and Johnson 1996; Kimura et al. 1997; Kenyon et al. 1993). In

primates, increasing age leads to broad changes in gene expression patterns, and caloric restriction seems to reverse these changes and leads to increased lifespan (Lee et al. 1999, 2000; Kayo et al. 2001; Weindruch et al. 2001).

The most relevant aging mechanism related to the work presented here is that of cellular or replicative senescence. In culture, human fibroblasts undergo a period of rapid proliferation followed by a period of a decline in growth, ultimately resulting in a complete cessation of cell division (senescence; Hayflick and Moorhead 1965). Senescent cells do not die, and they can be maintained in culture for long periods of time (Bayreuther et al. 1988). Senescence is inevitable for normal somatic cells, provided they do not acquire changes such as abnormal chromosome number (aneuploidy). Importantly, it is the number of divisions that determines cellular lifespan, not the actual time spent in culture, indicating that the cells have a mechanism for counting their divisions (Hayflick 1985). In addition, cells in the senescent stage behave differently than proliferating cells in that they are less responsive to mitogens, exhibit changes in expression of growth factors and growth factor receptors, and show alterations in signal transduction (Troen and Cristafalo 2001; Calabrese et al. 2006; Bishop et al. 2007).

It has been clearly demonstrated that telomere shortening acts as a mechanism that regulates the division potential of a cell (Harley 1991). Telomeres are structures at the ends of vertebrate chromosomes that protect against degradation and fusion with other chromosomes (Greider 1990). Telomere length shortens with each cell division; experimental elongation of telomeres extends the lifespan of cells (Wright et al. 1996), and introduction of the active telomerase enzyme prevents senescence in human cells (Bodnar et al. 1998). The telomerase enzyme, which is minimally composed of an integral RNA component (human telomerase RNA) and an enzymatic polymerase subunit (human telomerase reverse transcriptase (hTERT)), is expressed in immortalized and germ line cells and functions to maintain telomere length (Counter et al. 1994; Sugihara et al. 1996). Telomere erosion in normal somatic cells is a primary cause of replicative senescence, but it is unlikely that it is the only causal factor. The focus of this work is to elucidate how replicative age in cells affects their ability to mount the classic heat shock response (HSR) in order to deal with environmental stress.

Discovered in 1962, the HSR is now known to exist in all organisms (Ritossa 1962). The HSR involves the expression of a family of proteins known as the heat shock proteins (HSPs; Brown et al. 2007). These proteins are highly conserved, have been shown to protect against protein aggregation, solubilize loose protein aggregates, assist in nascent protein folding, refold misfolded proteins, and sequester and target damaged proteins for degradation

(Lindquist and Craig 1988; Hightower 1991; Moseley 1997). In addition, they are involved in the formation of the telomerase enzyme complex (Holt et al. 1999a; Forsythe et al. 2001; Keppler et al. 2006) and function to confer cytoprotection and thermotolerance after mild stress in many organisms (Soti and Csermely 2002). Various stresses, including but not limited to heat, anoxia, hypoxia, ethanol, and heavy metal ions, induce the expression of HSPs (Lindquist and Craig 1988; Brown et al. 2007). These HSPs also serve as molecular chaperones and are most likely involved in numerous other normal cellular functions (Welsh 1993; Li and Thiele 2007). The HSPs are divided based on their size and function, with the major HSPs ranging from ~15 to 110 kDa and are primarily found in the cytosol, mitochondria, endoplasmic reticulum, and the nucleus.

The functions of the HSPs are widespread; here, we wish to focus primarily on their roles in the aging of mammalian cells. It has been demonstrated that older tissues have an attenuated HSR and that younger tissues can survive more stressful conditions. Therefore, it seems somewhat intuitive that decreased HSR is either a cause or a consequence of aging since the HSR is involved in protein turnover and stability, both of which are impaired with age. A decrease in HSR could lead to damaged protein aggregation, failure of ubiquitination of these aggregates and decreased Hsp90, a modulator of the proteasome, ultimately leading to proteasomal inhibition in aging tissues. In order to examine the effects of replicative aging on the HSR, we used the heavy metal salts zinc and stannous chloride to induce the HSR in cells of various replicative age.

Stannous (also known as tin) chloride and zinc chloride have been shown to induce the HSR (Lindquist and Craig 1988). Treatment with stannous chloride or zinc chloride induces Hsp70 and heme oxygenase in rats, which confers a cytoprotective state (Kappas and Maines 1976; House et al. 2001). Other reports suggest a differing pattern of chaperone induction by stannous and zinc in hepatoma cells (Mitani et al. 1993; Ghattas et al. 2002). In addition, transformed B lymphocytes from aged patients show a similar pattern of HSR as cells taken from young individuals (Ambra et al. 2004). Interestingly, most of the studies have used either immortalized cells or tumor-derived cell lines to investigate the stress response to these heavy metal salts, while the induction of the HSR via these metals in human fibroblasts has not been demonstrated. It is therefore the goal of this work to assess the cellular effects of these metals on human fibroblasts and to determine the overall effects of replicative aging on the HSR. We demonstrate here that treatment of normal cells with heavy metal salts causes an upregulation of HSP expression, and the extent of this upregulation is attenuated in older cells. Furthermore, we demonstrate that cells actively expressing the hTERT component of telomerase respond to heavy

metal salts with a robust HSR similar to normal fibroblasts, despite their increased replicative age, whereas cells lacking hTERT fail to mount an HSR as they approach senescence.

Materials and methods

Chemicals

Stannous chloride (CAS 10025-69-1) and zinc chloride (CAS 7646-85-7) were purchased from Sigma. Stock cultures were diluted in dH_2O to concentrations of 1 and 0.1 M for stannous and zinc chloride, respectively, and were stored at $-20^{\circ}C$.

Cell culture and treatments

BJ foreskin fibroblasts and retrovirally infected BJ-hTERT (Forsythe et al. 2002) cells were cultured in D9C media (DMEM (Invitrogen) with Medium 199 (Invitrogen) at a 4:1 ratio with 10% Cosmic Calf Serum (HyClone Laboratories, Inc.), and gentamicin (Invitrogen)) and incubated in 5% CO_2 . Untreated cells were at various population doublings (PD 23, PD 44, PD 69, hTERT PD 138). BJ (PD 23, PD 44, PD 69) and BJ-hTERT (PD 138) cells were exposed to stannous (tin) chloride (100 μM to 2 mM) or zinc chloride (10 to 200 μM) or no metal for 3 h. Cells were harvested and counted in triplicate at time points (3, 6, 12, 24 h, 4, 7 days) to determine growth effects of both metals. Harvested cells were used for western analysis.

Western analysis

Treated or untreated (0 time point) cells were harvested at 60–80% confluency and lysed with radioimmunoprecipitation buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% sodium dodecyl sulfate (SDS)). Lysates were clarified by centrifugation at $14,000\times g$ for 20 min. Protein was quantified using the bovine serum albumin protein quantification kit (BioRad); samples were electrophoresed on 12% SDS-polyacrylamide gel electrophoresis, and protein was transferred to nitrocellulose (BioRad). Membranes were blocked in phosphate-buffered saline (PBS) with 0.1% Tween 20 and 5% milk and then probed with monoclonal antibodies to Hsp90, Hsp70, Hsp40, p23 (all generous gifts of Dr. David Toft, Mayo Clinic, Rochester, MN, USA), and β -actin (Sigma) as previously described (Akalin et al. 2001).

Telomere length analysis

Telomere length was determined using the terminal restriction fragment (TRF) analysis. DNA was isolated from cells

using genomic tips (Qiagen, Santa Clarita, CA, USA), digested with a mixture of *Hinf*I, *Alu*I, and *Rsa*I restriction enzymes (Invitrogen) and resolved on a 0.7% agarose gel. The DNA ladder and G-rich telomere probe (TTAGGG)₄ were labeled with [γ -³²P]ATP (6,000 Ci/mmol) with unincorporated label removed from the reaction using the QIAquick nucleotide removal kit (Qiagen). The dried agarose gel was subjected to in-gel hybridization with the labeled probe, washed repeatedly with varying concentrations of SSC buffer, and exposed to a PhosphorImaging cassette for 24 h. Average telomere length was calculated as previously described (Elmore et al. 2002).

Results

In order to determine the effects of replicative and physiological age on the induction of the HSR, we employed the use of four groups of cells. The first three groups consisted of normal BJ fibroblasts at different replicative ages, representing “young” [PD 20–30], “middle-aged” [PD 40–50], and “near senescent” [PD 68+]. In addition, BJ cells expressing the hTERT component of telomerase, which immortalizes BJ cells by activating telomerase and maintaining telomere length, were also tested. Figure 1 illustrates this difference in telomere length via TRF analysis. For cells in the “young” range, median telomere length was around 9 kb. As expected, as cells continue to divide, telomere length decreases (median lengths of 8 kb for PD 49 and 7 kb for PD 75). Cells expressing hTERT, however, maintain telomere length at 9 kb despite having over 150 PDs. Interestingly, the BJ-hTERT cells, while not having a significantly different median telomere length, showed a remarkable lack of heterogeneity (absence of the shortest telomeres as compared to the non-hTERT-expressing cells, Fig. 1), suggesting that telomerase is capable of maintaining the shortest telomeres without a net gain in global telomere length (Hemann et al. 2001). We hypothesized that BJ-hTERT cells, while being “older” in a replicative sense, would behave as “younger” cells physiologically, due to their ability to maintain telomere length.

Many heavy metals are toxic at high concentrations. Therefore, it was necessary to determine what concentrations would produce only a “nontoxic” stress in human fibroblasts. In order to determine the optimal acute dose and minimal toxic dose for both metals, normal fibroblast cells (PD 23) were acutely exposed to increasing concentrations of stannous chloride (100 μM to 3 mM) or zinc chloride (10 to 300 μM) for 3 h, washed with $1\times$ PBS, and incubated with untreated media. Maximum survivable concentrations were determined by manual counting of cells after 5 days, and harvested pellets were stored at $-80^{\circ}C$ for

Fig. 1 Decreased telomere length with age in human fibroblasts. Cultured diploid human fibroblasts, BJ, at young (PD 26), middle (PD 49), and old (PD 75) age, as well as hTERT-infected cells (PD 158) were tested for telomere length using the TRF protocol as previously described (Elmore et al. 2002). The white bars indicate the median telomere length for each sample. Of note is the relative heterogeneity in the samples without hTERT, suggesting that telomerase is capable of elongating the shortest telomere in the cells (Hemann et al. 2001)

use in other assays. There was no observable difference in growth for SnCl_2 between 0 and 500 μM or for ZnCl_2 between 0 and 100 μM , while higher concentrations for both ($>200 \mu\text{M}$ ZnCl_2 or $>2 \text{ M}$ SnCl_2) showed severe toxicity and cell death (data not shown). In addition, we assessed the growth rates for both heavy metal salts using the fibroblasts at the “young,” “middle,” and “old” replicative ages. The growth rates after an initial growth arrest (12–24 h) of the “young” and “middle” age cells were minimally affected by nontoxic concentrations, while the “old” cells undergo a growth arrest and ultimately fail to resume division after a week in culture (data not shown).

In addition to growth as a measure for recovery from stress, the expression levels of a variety of chaperones (Hsp90, Hsp70, Hsp40, and p23) were also assessed to demonstrate the effects of stannous and zinc chloride on normal fibroblasts at PD 23 (Fig. 2), which were acutely treated with two concentrations of both zinc (10, 100 μM) and stannous (50, 500 μM) chloride for 3 h. The cells were allowed to recover in media without the chloride salts for the indicated times and subsequently harvested for Western analysis to detect the HSR chaperone induction. In Fig. 2a, both lower concentrations of heavy metal salts induced a robust induction of chaperone expression within 3 h after treatment, and levels of chaperone expression return to normal within 24 h. The control of HSP expression is typically through the heat shock factor (HSF)-1 transcription factor (Zou et al. 1998), and although p23 is not a known target of HSF-1, it is still upregulated together with the HSPs. While there is a similar robust induction of chaperone expression at higher salt concentrations, chaperone expression returns to normal levels within 96 h rather than 24 h (Fig. 2b). Importantly, treated cells continue to divide after incubation with the heavy metal salts, as stated above.

We then determined the effects of stannous and zinc chloride on fibroblasts with an increased replicative life-span (i.e., PD 44; Fig. 3). Again, cells were acutely treated

BJ fibroblasts PD23

Fig. 2 Robust recovery of chaperone expression in young (PD 23) fibroblasts. BJ fibroblasts were exposed to low (a) or high (b) concentrations of stannous (SnCl_2) and zinc (ZnCl_2) chloride for 3 h and were allowed to recover in fresh serum containing media for the indicated times, followed by Western analysis. Quantification below each lane was done by densitometry for a minimum of two separate blots, with the untreated lane set to 1

Fig. 3 Decreased recovery of chaperone expression from heavy-metal-induced stress in middle-aged (PD 44) fibroblasts. BJ fibroblasts were exposed to low (panel a) or high (panel b) concentrations of stannous and zinc chloride for 3 h and were allowed to recover in fresh serum containing media for the indicated times, followed by Western analysis. Quantification below each lane was done by densitometry for a minimum of two separate blots, with the untreated lane set to 1

at the indicated concentrations and allowed to recover for the indicated times. Western analysis demonstrates that at this age (PD 44), cells are again able to induce the HSR within 3 h of treatment with both heavy metal salts at the two indicated concentrations. At the lower concentrations (Fig. 3a), cells appear to maintain an elevated level of chaperone expression well beyond the 24-h mark, returning to basal levels after 96 h. The higher concentrations (Fig. 3b) induced an even longer maintenance of elevated chaperone levels, requiring the full 7 days of recovery time to return to basal levels. After chaperone levels returned to normal, the PD 44 cells resumed a normal division pattern in culture at all concentrations tested, as stated above for the PD 23 cells.

We next repeated the same procedure on fibroblasts that were approaching senescence (PD 69; Fig. 4). At the lower concentrations (Fig. 4a), chaperone expression was induced but to a much smaller degree than in the younger cells, while there appears to be no significant difference in basal (untreated) levels of chaperones between young, middle, and older populations (compare untreated lanes in Figs. 2a,b, 3a,b, and 4a,b). Additionally, chaperone levels failed to return to normal after the 7-day recovery time. The higher concentrations (Fig. 4b) produced the same pattern of induction, again with failure to return to basal levels by the

7-day recovery time. Furthermore, treated cells demonstrate a variety of morphological changes including swelling and detachment, most of which ultimately fail to divide when passaged further. These results indicate that as cells approach senescence, the ability to mount a robust HSR is compromised, and cells are subsequently unable to recover from the stress.

Because telomerase-expressing fibroblasts appear to behave like young normal cells (Forsythe et al. 2002), we determined the effect of heavy metal salts on fibroblasts that express the hTERT component of telomerase. These BJ-hTERT cells divide well beyond the point of senescence of normal BJ cells (Bodnar et al. 1998; Forsythe et al. 2002) and appear phenotypically normal. The BJ-hTERT cells at a replicative age of 118 PDs were treated with the indicated concentrations of heavy metal salts and chaperone expression levels were determined by Western (Fig. 5a,b). At all four concentrations of heavy metal salts in Fig. 5a,b, a robust HSR was induced by 3 h, chaperone levels returned to normal by 24 h, and the cells resumed normal cell growth, all of which was nearly identical to that observed for the “young” fibroblasts (PD 23). This indicates that the BJ-hTERT cells, despite being nearly 100 PDs older than the young fibroblasts, behave physiologically similar to the young diploid BJ cells. To this end, we compared the chaperone expression levels of both

Fig. 4 Lack of recovery of chaperone expression from stress in aged (PD 69) fibroblasts. BJ fibroblasts were exposed to low (a) or high (b) concentrations of stannous and zinc chloride for 3 h and were allowed to recover in fresh serum containing media for the indicated times, followed by Western analysis. Quantification below each lane was done by densitometry for a minimum of two separate blots, with the untreated lane set to 1

Fig. 5 Telomerase-expressing fibroblasts show enhanced recovery from stress. BJ fibroblasts were infected with hTERT and selected with puromycin as previously described (Forsythe et al. 2002). Cells were treated with identical concentrations of heavy metal salts as in Fig. 2, as well as elevated doses (2 M SnCl₂ and 200 μ M SnCl₂). As before, levels of heat shock proteins were determined by Western analysis and compared to young cells at PD 23 at the highest concentrations of salt. Quantification below each lane was done by densitometry for a minimum of two separate blots, with the untreated lane set to 1

“young” BJ cells (PD 23) and BJ-hTERT (PD 118) cells in response to near-toxic concentrations of heavy metal salts (2 M SnCl₂ and 200 μ M ZnCl₂). BJ-hTERT cells (Fig. 5c) and young BJ cells (PD 23; Fig. 5d) were acutely treated with the indicated concentrations of salts and allowed to recover. For the BJ-hTERT cells, a robust induction of chaperone expression was observed within 3 h followed by reduction to basal levels by 96 h (Fig. 5c). Normal fibroblasts (PD 23) responded similarly with an HSR (Fig. 5d) with only a modest difference in recovery time in terms of chaperone levels. Importantly, for the near-toxic concentrations, both cell types continued to divide following 7 days of recovery, although at a slightly reduced rate (data not shown). These results indicate that BJ-hTERT cells are able to induce a robust HSR and recover as well as, if not better than, the “young” BJ cells, despite having a significantly elevated lifespan. Taken together, our data suggest a strong correlation between telomere length and physiological age and that cells that are maintaining telomeres are locked in a physiologically “young” state, *vis a vis* with an ability to react to and recover from environmental stress.

Discussion

The effects of environmental stress on the cellular aging process are of great interest. Every species induces the HSR after exposure to stressful conditions, and this response confers a cytoprotective state that enables the organism to endure a subsequent, potentially lethal, stress. As organisms age, their ability to cope with environmental stress diminishes, as does their ability to induce the HSR. Here,

we sought to examine the effects of heavy metal salts on human fibroblasts as related to HSP expression. We have demonstrated that acute treatment with zinc or stannous chloride induces the HSR in a dose-dependent manner and that the replicative age of normal cells determines the magnitude of this HSR. Furthermore, cells expressing active telomerase do not attenuate their HSR with overall replicative age.

The heavy metal salts were chosen for their diverse cellular effects, representing a range of cellular and physiological changes when exposed to tumor and normal cells. Nutritionally, zinc is a critical component of a variety of cellular functions and pathways, many of which are important for cell survival (Sunderman 1995). In fact, ZnCl₂ has been shown to have antiapoptotic properties in lymphoid, tumor, and normal cells (Sunderman 1995). However, excessive zinc can alter the steady-state levels of other important vitamins and minerals, contributing to disruption of the numerous metabolic processes in which zinc is involved. The effects of SnCl₂ (also known as tin) on physiology are not well described, although studies suggest that elevated tin may negatively effect the immune system (Arakawa 1997) and accelerate neuronal aging (Candura et al. 2000). In the context of our results, continued exposure of cells in the body to excessive levels of these heavy metal salts will likely contribute to and/or cause chronic diseases, possibly related to premature aging phenotypes.

The HSR response has been shown to be mediated by the release of the main heat shock transcription factor, HSF1, which is bound by Hsp90 and held in the cytoplasm as a monomer (Ali et al. 1998). When cells are stressed by heat, high salt, or other environmental changes, Hsp90

releases HSF1, which forms a functional trimer for transcriptional activation of chaperone genes (Hsp90, Hsp70, Hsp27, etc.; Zou et al. 1998). To our knowledge, there is no evidence for HSF1 regulating the transcriptional upregulation of the cochaperone, p23, yet we observe consistent and significant increases in p23 during the HSR. Mechanistically, it is likely that p23 is not transcriptionally altered but posttranslationally stabilized in its association with Hsp90. Because there is more Hsp90 being expressed, it is expected that there would be more p23 necessary to functionally assemble and fold protein complexes, which would ultimately cause an increase in the stability of the p23 protein. Alternatively, it is conceivable that p23 is upregulated independently of Hsp90-induced stability, perhaps via a non-HSF-1-mediated transcriptional upregulation or post-translational modification. We are currently investigating these mechanisms.

To assess the effect of chronological age on the induction of the HSR, we used four different populations of cells at varying replicative ages and exposed them to varying concentrations of both metals. These cells were permitted to recover for up to 7 days before they were harvested in order to establish a temporal profile of HSP expression for each population. Growth rate and heat shock protein expression were assessed as surrogate markers of the HSR after treatment. We chose four proteins to monitor Hsp90, Hsp70, Hsp40 (not shown), and the cochaperone p23. These proteins were selected based on their previously established role in the HSR and in protein folding and assembly. The youngest cells induced HSP levels, which returned to basal levels by 7 days, even at the highest concentrations of metals. These “young” cells were able to recover from the stress and return to normal growth under all conditions tested. The “middle”-aged population of cells (PD 44) was also able to induce the HSR in response to all concentrations of metals, yet took substantially longer to both exit growth arrest and to bring HSP levels back to normal, particularly at the higher concentrations. Even so, these “middle”-aged cells were capable of returning to their normal growth patterns with nearly identical timing as the youngest cells (PD 23). The effects of the HSR were even more pronounced in the older, near-senescent cells (PD 69). At low and mid concentrations of both metals, the “old” cells were able to induce some HSP protein expression, but the HSP levels remained elevated and did not return to normal even after 7 days. The higher concentrations of metals, tolerable in the younger populations, proved too toxic for the older cells, as both overall amount of cellular protein and cell number were greatly reduced. These patterns are indicative of the effects of chronologic age in tissues; the more divisions the cells have gone through, the more difficult it is to recover from stress (Hayflick 1985; Rubin 2002).

Interestingly, normal cells expressing hTERT, the catalytic component of telomerase, avoid these age-associated effects and are able to both induce the HSR and recover just as the younger populations of cells, despite the fact that these cells are chronologically older than the near-senescent cells. It has been demonstrated previously that hTERT expression results in the activation of the telomerase enzyme, which maintains telomere length in human fibroblasts (Bodnar et al. 1998). Telomere maintenance by telomerase in normal cells allows for an extended lifespan by compensating for the end replication problem, ultimately resulting in the avoidance of senescence. This maintenance of genomic integrity appears to render these cells more resistant to stress even at a much higher PD. These data parallel studies done previously on normal and tumor cells with and without telomerase expression, where cells were exposed to conditions that induce apoptosis and telomerase activity (hence, telomere maintenance) conferred some resistance to growth-factor-induced programmed cell death (Holt et al. 1999b; Fu et al. 1999). It is likely that a functional stress response in the context of telomere maintenance is independent of chronological age and that physiologic age may be a better indicator of the HSR. Either way, it is clear that as normal fibroblasts continue to divide, their potential to mount a successful HSR is diminished; however, if the “internal telomere clock” is switched off by activating telomerase, cells continue to respond adequately. Taken together, the results presented here offer new insights into a role for telomere function and cellular aging, providing evidence for how aging cells differentially respond to environmental stresses.

References

- Akalin A, Elmore LW, Forsythe HL, Amaker BA, McCollum ED, Nelson PS, Ware JL, Holt SE (2001) A novel mechanism for chaperone-mediated telomerase regulation during prostate cancer progression. *Cancer Res* 61:4791–4796
- Ali A, Bharadwaj S, O'Carroll R, Ovsenek N (1998) Hsp90 interacts with and regulates the activity of heat shock factor 1 in *Xenopus* oocytes. *Mol Cell Biol* 18:4949–4960
- Ambra R, Mocchegiani E, Giacconi R, Canali R, Rinna A, Malavolta M, Virgili F (2004) Characterization of the hsp70 response in lymphoblasts from aged and centenarian subjects and differential effects of in vitro zinc supplementation. *Exp Gerontol* 39:1475–1484
- Arakawa Y (1997) Biological activity of tin and immunity. (Japanese) *Sangyo Eiseigaku Zasshi* 39:1–20
- Bayreuther K, Rodemann HP, Hommel R, Dittmann K, Albiez M, Franz PI (1988) Human skin fibroblasts in vitro differentiate along a terminal cell lineage. *Proc Natl Acad Sci U S A* 85:5112–5116
- Bishop SC, Burlison JA, Blagg BS (2007) Hsp90: a novel target for the disruption of multiple signaling cascades. *Curr Cancer Drug Targets* 7:369–388
- Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu C-P, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE (1998)

- Extension of lifespan by introduction of telomerase into normal human cells. *Science* 279:349–352
- Brown MA, Zhu L, Schmidt C, Tucker PW (2007) Hsp90—from signal transduction to cell transformation. *Biochem Biophys Res Commun* 363:241–246
- Calabrese V, Butterfield DA, Scapagnini G, Stella AM, Maines MD (2006) Redox regulation of heat shock protein expression by signaling involving nitric oxide and carbon monoxide: relevance to brain aging, neurodegenerative disorders, and longevity. *Antioxid Redox Signal* 8:444–477
- Candura SM, Butera R, Gandini C, Locatelli C, Tagliani M, Fasola D, Manzo L (2000) Occupational poisoning with psychiatric manifestations. (Italian) *G Ital Med Lav Ergon* 22:52–61
- Counter CM, Hirte HW, Bacchetti S, Harley CB (1994) Telomerase activity in human ovarian carcinoma. *Proc Natl Acad Sci U S A* 91:2900–2904
- Elmore LW, Rehder CW, Di M, McChesney PA, Jackson-Cook CK, Gewirtz DA, Holt SE (2002) Adriamycin-induced replicative senescence in tumor cells requires functional p53 and telomere dysfunction. *J Biol Chem* 277:35509–35515
- Failla G (1958) The aging process and carcinogenesis. *Ann NY Acad Sci* 71:1124–1140
- Finch CE, Tanzi RE (1997) Genetics of aging. *Science* 278:407–411
- Forsythe HL, Jarvis JL, Turner JW, Elmore LW, Holt SE (2001) Stable association of Hsp90 and p23, but not Hsp70, with active human telomerase. *J Biol Chem* 276:15571–15574
- Forsythe HL, Jensen KO, Elmore LW, Holt SE (2002) Retroviral-mediated expression of telomerase in normal human cells provides a selective growth advantage. *Int J Oncol* 20:1137–1143
- Fu W, Begley JG, Killen MW, Mattson MP (1999) Anti-apoptotic role of telomerase in pheochromocytoma cells. *J Biol Chem* 274:7264–7271
- Ghattas MH, Chuang LT, Kappas A, Abraham NG (2002) Protective effect of HO-1 against oxidative stress in human hepatoma cell line (HepG2) is independent of telomerase enzyme activity. *Int J Biochem Cell Biol* 34:1619–1628
- Greider CW (1990) Telomeres, telomerase, and senescence. *Bioassays* 12:363–369
- Harley CB (1991) Telomere loss: mitotic clock or genetic time bomb? *Mutat Res* 256:271–282
- Hayflick L (1985) The cell biology of aging. *Clin Geriatr Med* 1:15–27
- Hayflick L, Moorhead PS (1965) The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res* 37:614–636
- Hemann MT, Strong MA, Hao LY, Greider CW (2001) The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* 107:67–77
- Hightower LE (1991) Heat shock, stress proteins, chaperones, and proteotoxicity. *Cell* 66:191–197
- Holt SE, Aisner DL, Baur J, Tesmer VM, Dy M, Ouellette M, Trager JB, Morin GB, Toft DO, Shay JW, Wright WE, White MA (1999a) Functional requirement of p23 and Hsp90 in telomerase complexes. *Genes Dev* 13:817–826
- Holt SE, Glinsky VV, Ivanova AB, Glinsky GV (1999b) Resistance to apoptosis in human cells conferred by telomerase function and telomere stability. *Mol Carcinog* 25:241–248
- House SD, Guidon PT Jr, Perdrizet GA, Rewinski M, Kyriakos R, Bockman RS, Mistry T, Gallagher RA, Hightower LE (2001) Effects of heat shock, stannous chloride, and gallium nitrate on the rat inflammatory response. *Cell Stress Chap* 6:164–171
- Jazwinski SM (1996) Longevity, genes, and aging. *Science* 273:54–59
- Kappas A, Maines MD (1976) Tin: a potent inducer of heme oxygenase in kidney. *Science* 192:60–61
- Kayo T, Allison DB, Weindrich R, Prolla TA (2001) Influences of aging and caloric restriction on the transcriptional profile of skeletal muscle from rhesus monkeys. *Proc Natl Acad Sci U S A* 98:5093–5098
- Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366:461–464
- Keppeler BR, Grady AT, Jarstfer MB (2006) The biochemical role of the heat shock protein 90 chaperone complex in establishing human telomerase activity. *J Biol Chem* 281:19840–19848
- Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G (1997) daf-2, an insulin receptor-like gene that regulates longevity and diapause in *C. elegans*. *Science* 277:942–946
- Lee CK, Klopp RG, Weindrich R, Prolla TA (1999) Gene expression profile of aging and its retardation by caloric restriction. *Science* 285:1390–1393
- Lee CK, Weindrich R, Prolla TA (2000) Gene expression profile of the ageing brain in mice. *Nat Genet* 25:294–297
- Levine RL, Stadtman ER (1996) Protein modifications with aging. In: Schneider EL, Rowe JW (eds) *Handbook of the biology of aging*. Academic, San Diego, pp 184–197
- Li Z, Thiele CJ (2007) Targeting Akt to increase the sensitivity of neuroblastoma to chemotherapy: lessons learned from the brain-derived neurotrophic factor/TrkB signal transduction pathway. *Expert Opin Ther Targets* 11:1611–1621
- Lindquist S, Craig EA (1988) The heat shock proteins. *Ann Rev Genet* 22:631–677
- Mitani K, Fujita H, Fukuda Y, Kappas A, Sassa S (1993) The role of inorganic metals and metalloporphyrins in the induction of haem oxygenase and heat-shock protein 70 in human hepatoma cells. *Biochem J* 290(Pt 3):819–825
- Moseley PL (1997) Heat shock proteins and the adaptation of the whole organism. *J Appl Physiol* 83:1413–1417
- Murakami S, Johnson TE (1996) A genetic pathway conferring life extension and resistance to UV stress in *Caenorhabditis elegans*. *Genetics* 143:1207–1218
- Ritossa F (1962) A new puffing pattern induced by temperature shock and DNP in *Drosophila*. *Experientia* 18:571–573
- Rubin H (2002) The disparity between human cell senescence in vitro and lifelong replication in vivo. *Nat Biotechnol* 20:675–681
- Sohal RS, Weindrich R (1996) Oxidative stress, caloric restriction, and aging. *Science* 273:59–63
- Soti C, Csermely P (2002) Chaperones and aging: role in neurodegeneration and in other civilizational diseases. *Neurochem Int* 41:383–389
- Sugihara S, Mihara K, Marunouchi T, Inoue H, Namba M (1996) Telomere elongation is observed in immortalized human fibroblasts by treatment with ⁶⁰Co gamma rays or 4-nitroquinoline 1-oxide. *Human Genet* 97:1–6
- Sunderman FW (1995) The influence of zinc on apoptosis. *Ann Clin Lab Sci* 25:134–142
- Troen BR, Cristafalo VJ (2001) Cell and molecular aging. In: Rosenthal RA, Zenilman ME, Katlic MR (eds) *Principles and practice of geriatric surgery*. Springer, New York, pp 8–23
- Weindrich R, Kayo T, Lee CK, Prolla TA (2001) Microarray profiling of gene expression in aging and its alteration by caloric restriction in mice. *J Nutr* 131:918S–923S
- Welsh WJ (1993) How cells respond to stress. *Sci Am* 268:56–64
- Wright WE, Brasiskyte D, Piatyszek MA, Shay JW (1996) Experimental elongation of telomeres extends the lifespan of immortal X normal cell hybrids. *EMBO J* 15:1734–1741
- Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R (1998) Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell* 94:471–480